

## **REMARKS**

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and these comments.

### **I. Status of the Claims**

With this submission, claim 1 is amended to incorporate salient recitation from claims 4, 6, and 7, which are canceled without prejudice or disclaimer. Support for these amendment can be found throughout the specification. In particular support for the term “healthy individual” can be found in Example 2 on pages 14-15 of the specification. Claim 8 is also amended. No claims are newly added. Upon entry of this paper, therefore, claims 1-3, 5, 8 and 9 will remain pending and under consideration.

The cancellation of claims does not constitute acquiescence in the propriety of any rejection set forth by the Examiner. Applicants reserve the right to pursue the subject matter of the canceled claims in subsequent divisional applications.

### **II. Submission of Art for Examiner’s Consideration**

The examiner refused consideration of several proffered publications, due to an alleged non-compliance with 37 CFR 1.98(a)(2). Applicants provide new, high-quality copies of the publications in question, as listed in a new PTO/SB/08 form, for the examiner’s formal consideration.

### **III. Objections to the Specification**

The Office objects to the specification for using legal phraseology such as “means” and “said.” Additionally, the Office objects to the typographical error found in the Heading “Example 2” on page 10.

Applicants have edited the abstract to remove the legal phraseology. Additionally, applicants have corrected the typographical error to change “Example 2” to “Example 1.” As such, applicants believe the objection is moot.

### **IV. Claim Rejection - 35 U.S.C. §103 - Waki in view of Okamoto**

Claims 1-9 stand rejected over Waki *et al.*, *Am. J. Pathology* 161: 399-403 (2002), in view of Okamoto *et al.*, *Proc. Nat'l Acad. Sci. USA* 94: 5367-71 (1997). The Office admits that “Waki does not teach a method of quantitatively determining the frequency of epimutation of a particular gene in said population of cells” (Office Action, page 6). Additionally, the Office acknowledges that “Waki does not teach a method wherein the normal tissue is normal peripheral blood” (*id.*). The Office looks to Okamoto, however, to cure these deficiencies. Applicants respectfully traverse the rejection.

#### A. Overview of the Invention

The presently claimed methodology relates to assessing the presence of germ-line or early epigenetic mutations in a tumor suppressor gene, where the silencing of the tumor suppressor is associated with cancer. It follows that a germ-line or an early epigenetic mutation present in one allele tumor suppressor renders the cell harboring the mutation prone to develop into a cancer cell, due to the fact that a subsequent somatic mutation in the second allele may lead to loss-of-function in the affected cell.

Tumor suppressor have been known for decades. It also is well-known that a germ line mutation, such as gross or minor deletions or point mutations, manifests in an significant increased risk of development of cancer, such as retinoblastoma, in case a germ line mutation in Rb, and breast cancer, in case a germline mutation in BRAC1/2).

The present inventors identified an allele of the hMLH1 carrying a G/A polymorphism. Most surprisingly, the inventors discovered that the tumor suppressor hMLH1, harboring the G polymorphism, is particularly susceptible to epimutation by DNA methylation. In contrast, the hMLH1 A allele was never found to be hypermethylated. (See data described in the application on page 12, last paragraph, and on page 13.) Thus, the probability of detecting epimutated hMLH1 alleles increases significantly if the sample is obtained from a subject carrying at least one hMLH1 G allele compared to a non affected individual being homozygous for the hMLH1 A allele. Based on the limit of sensitivity of the COBRA hybrid PCR assay, for example, the inventors estimated that individual TT carries between 1 in 500 to 1 in 1000 methylated alleles (page 13, lines 23 - 33).

Apart from genes that are subject to normal parent-of-origin-specific expression, applicants conceived that the hMLH1 G allele, as described in the present application, is illustrative of a category of allele that is prone to epimutation by DNA methylation. Part and parcel of this conception is the notion that affected tumor suppressor alleles may be particularly susceptible to hypermethylation, which also manifests in normal tissues of carriers, as in the case of hMLH1.

Pursuant to the invention as conceptualized, there are two distinctions that set the invention apart from all of the prior art. The key distinction over the cited references is the notion that “risk of cancer” is determined from a “**healthy**” individual. As can be seen from Example 2 of the specification, a “**healthy individual**” is an individual who has **yet to manifest any pathology**. In contrast, the cited art focuses on individuals who present oncological scintilla (hence, are not “**healthy**”) and on diagnoses concerning the risk that the extant pathology will spread.

A second notable feature of the subject invention resides in its **distal** nature, perhaps most evident in the recitations of claim 2. Pursuant to the invention, that is, the risk of cancer can be assessed by analyzing normal tissue even though the cancer may manifest in another tissue. Thus, the inventors were the first to describe the use of DNA methylation in tissues distal (situated away) from the tissue affected by a risk of manifesting the disease; *e.g.*, assessing the risk of cancer in colon tissue by use of peripheral blood (the distal tissue).

**B. No Reasonable Permutation of Teachings Gleaned from Waki and Okamoto Can Render the Claims Obvious per Section 103**

**1. Waki only tests tissue that is not “normal” but rather is affected by pathology, in individuals who are not “healthy”**

Waki has proposed that “detection of hMLH1 methylation in nonneoplastic gastric epithelia may be a useful screening method for identifying individuals who may be at risk of developing gastric cancer” (page 403). With respect to such “non-neoplastic” tissue, however, Waki clearly indicates that the “risk” of developing cancer related to MLH1 methylation lies in the fact that *carcinogenesis already is underway* in the gastric epithelia of the tested subjects.

Specifically, Waki “reasoned that this phenomenon during aging may be an early warning signal for the beginning of cancer in the gastric epithelia” (page 399; emphasis added).

Furthermore, all of Waki’s disclosures point to the fact that the prior-art method was directed solely to early detection / early signs of disease. Thus, Waki states that the reported “results suggest that methylation of hMLH1 in nonneoplastic gastric epithelia is pathological and may serve as a useful marker for gastric cancer development” (page 402; emphasis added).

“Pathological” in this context is not “normal,” as the latter term is employed in the present claims. That is, Waki only deals with the diagnosis of the early stages of cancer, *i.e.*, when cancer is already present. In contrast, the methodology of the claimed invention the diagnosis of the risk of disease in a healthy (*i.e.*, non-pathological) tissue. This distinction between Waki and the claimed invention cannot be remedied by Okamoto.

## 2. Waki does not test distal tissue

Furthermore, Waki only examined the tissue in which the cancer arose. By contrast, applicants’ approach may entail examining tissues distal (*i.e.*, situated away) from a tissue affected by a risk of manifesting the disease. Thus, the presently recited “normal tissue” can be taken from blood, from hair, or from the buccal cavity and the risk then determined for cancer elsewhere, for example, colon, breast, or testicular cancer.

For instance, Waki examined **gastric tissue** to test for **gastric cancer**. In fact, when Waki tested other tissues the “methylation of hMLH1 was not found in any organ in all age groups except for a nonneoplastic lung tissue obtained from a 78-year-old male who died of lung cancer” (page 400). Again, Waki only suggested that the methylation status of **lung tissue** can be correlated with **lung cancer**. Compare present claim 2, for example, which prescribes testing of normal peripheral blood, normal hair follicle tissue, and normal tissue from the buccal cavity, respectively, to ascertain disease risk elsewhere.

## 3. Waki teaches away from use of distal tissue

One of ordinary skill would have been directed by Waki away from the presently claimed invention. That is, Waki shows that the methylation status of E-cadherin, hMLH1, and p16 was “not detected in nonneoplastic cells of any organs obtained from individuals who were 22 years

and younger" (page 400). When testing for the methylation status of hMLH1 in other organs, moreover, Waki observed that "[m]ethylation of hMLH1 was not found in any organ in all age groups except for a nonneoplastic lung tissue obtained from a 78-year old male who died of lung cancer." (*id.*). The one tissue where hMLH1 was found methylated was lung tissue, from a patient who died of lung cancer.

Again, this would not have suggested the prospect of garnering a methylation "signal" of disease risk from a tissue distal from the site of disease risk, as applicants' approach highlights (see claim 2).

Example 1 of the present application teaches that "12 of 22 healthy blood donors show some detectable level of hypermethylated hMLH1" (specification at page 15, lines 1-3). Additionally, applicants show that, "[f]or the p16 gene, 18 of 29 healthy blood donors showed a detectable level of hypermethylated alleles" (*id.*, lines 3-7). Importantly, applicants thus teach that "more cells in an individual that carry the epimutation, the higher will be that individual's risk of developing cancer.... the risk of developing cancer [therefore] may be assessed by measuring the proportion of somatic cells carrying a particular epimutation" (*id.*, lines 18-24).

This insight has no precedent in either Waki or Okamoto, and no reasonable combination of these references could have suggested it. Furthermore, as the Office Action correctly states on page 6, Waki says nothing about quantitative determination of the epimutation as it relates to any risk, *per se*, even the risk of carrying a tumor as opposed to that of developing one in the future.

#### 4. Okamoto cannot cure the defects of Waki

The Office cites to Okamoto for the proposition that "unaffected adjacent kidney and peripheral blood of Wilms tumor patients to determine whether aberrant methylation of H19 was present in normal tissues." Office Action, page 6.

At the outset applicants take note, even though the examiner has not, that Okamoto deals with Wilms tumor, which involves **an imprinted locus** (see discussion below). Yet, the present claims explicitly exclude a method focusing on imprinted genes, *i.e.*, those that have parent-of-origin-specific expression patterns. For this reason alone, the combination of Waki and Okamoto is an improper basis for the alleged *prima facie* case under Section 103.

Moreover, Okamoto himself cites Bestor *et al.*, *Nat. Genet.* 12: 363-67 (1996), to explain the mechanism behind the observed phenomenon, which involves an “imprinted” locus (see Okamoto at 5371, column 1). Bestor states that “[a]lles at imprinted loci are asymmetrically methylated and...will tend to convert asymmetrical allelic methylation patterns toward the more heavily methylated pattern” (page 366, column 1).

With this perspective, Bestor addresses the particular situation of imprinted loci associated with Wilms tumor:

Certain pathological human conditions show abnormalities in the functional imprinting of particular chromosomal regions which might arise via this type of interchromosomal transfer of epigenetic information. For example, **Wilms' tumors frequently show conversion** of a uniparental (bipaternal) methylation and expression pattern at imprinted loci in the H19/IGF2 region on chromosome 11p15.5, which could result from the **local transfer of methylation patterns from the paternal chromosome to the less heavily methylated maternal chromosome**.

*Id.* (emphasis added).

The present claims eschew “normal parent of origin-specific expression” precisely to avoid the disruption (“conversion”) of methylation pattern that Bestor describes. That is, a transfer of methylation patterns from the paternal chromosome to the less heavily methylated maternal chromosome, per Bestor, effectively obfuscates any risk estimate based on methylation status, pursuant to applicant’s claimed invention.

##### 5. Okamoto teaches away from the use of distal tissue

Regardless of whether imprinted genes are excluded from the claims, as discussed above, Okamoto does not stand for the proposition that the Office asserts. The crux of the Okamoto paper is an observed high prevalence of epigenetically modified cells among “normal” kidney tissue, close by a kidney cancer (Wilms tumor). Like Waki, therefore, Okamoto focuses on the tissue in which the cancer arose. In particular, Okamoto simply finds that “normal” kidney tissue that is adjacent to the Wilms tumor is susceptible to becoming cancerous. As noted above in the Waki discussion above, claim 2 captures this distality concept. As shown below, Okamoto actually teaches away from this concept.

The Office is correct that Okamoto examines peripheral blood. Yet, Okamoto **does not correlate the methylation status in peripheral blood with the risk of Wilms tumor.**

In fact, Okamoto shows quite the opposite. As the Office correctly notes, an increased H19 methylation was detected in only **one of four patients having tumors** with IFG-II LOI, *i.e.*, relaxation of IFG-II/H19 imprinting (page 5370 in Table 1, “case 2”). In other words, all four patients tested were known already to be positive for Wilms tumor, but only one came back positive when testing with peripheral blood.

Thus, Okamoto would afford a “correct” risk analysis, in relation to applicants’ claimed methodology, only 25% of the time from a population that should be 100% positive. This impression of Okamoto’s results, namely, that one would be better served, for risk assessment, by flipping a coin (50% accuracy) rather than running a blood test (25% accuracy), actually would have taught away from the claimed invention. Confounding these results even more is the fact that the one individual that tested positive was “previously reported to have gigantism and constitutional relaxation of IFG-II imprinting” (page 5368, column 1).

For at least these reasons, applicants respectfully request reconsideration and withdrawal of the rejection.

#### V. Claim Rejection - 35 U.S.C. §103 - Wong in view of Okamoto

Claims 1-8 stand rejected over Wong *et al.*, *Cancer Res.* 59: 71-73 (1999), in view of Okamoto. The Office looks to Okamoto to cure the admitted deficiency that “Wong does not teach ... quantitatively determining the frequency of epimutation of a particular gene in [a] population of cells” (Office action, page 10). Applicants respectfully traverse the rejection.

Wong deals with circulating tumor DNA. Wong’s method is non-quantitative and in no way suggestive of assessing disease risk in a normal individual. Accordingly, nothing from Okamoto could have bridged the gap between Wong and the presently claimed invention, as a sustainable Section 103 analysis would require.

#### VI. Claim Rejection - 35 U.S.C. §103 - Wong in view of Okamoto and Waki

Claims 9 is rejected over Wong in view of Okamoto and Waki. In light of the foregoing analysis, however, it is apparent that the skilled artisan would have had no inkling of the claimed

invention from any principled combination of these publications. To the contrary, their disparate disclosures would have taught away from that invention, as applicants have demonstrated with respect to Waki and Okamoto.

### CONCLUSIONS

Applicants submit that this application is in condition for allowance, and they request an early indication to this effect. Examiner Shaw is invited to contact the undersigned directly, should she feel that any issue warrants further consideration.

Respectfully submitted,

By S. A. Bent

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